Millie Firestone, MSD, ANL

Tom Irving, IIT

Randy Winans, CHM, ANL

Jin Wang, APS, ANL

Gopal Shenoy, APS,ANL

# Workshop Scope

- Membrane science is truly interdisciplinary.
- Advances in membrane science are scientifically and technologically exciting and significant.
- Areas of membrane science where X-ray characterization techniques are potentially useful:

  - Bio-Membranes
  - Organic-Membranes
  - Inorganic-Membranes
  - Micro and nanoscopic structure
  - Membrane surfaces and interfaces

Composites

# Workshop Objectives

1. Explore the breadth of science covered by the workshop topics, *not* limiting to synchrotron techniques alone.

2. Identify opportunities for continued scientific discovery and impact using the APS during the next 5-10 years in the interdisciplinary areas of membrane science.

3. Identify new scientific proposals/programs specific to the emerging areas of Membrane Science that the participants will bring to the APS during next 5 to 10 years. Also evaluate the capital and operational requirements for these proposals/programs.

4. In addition to available beamline capabilities at the APS, identify future needs to support research in this area of science and technology.

# Charge to the Participants

1. Identify science and technological problems in the field of membrane science to be addressed during the next 5-10 years using APS

2. Identify and justify the technical requirements to meet the grand challenge problems:

   - New instrumentation and techniques that need be developed on existing beamlines to perform new kind of science.
   - Need for new dedicated beamlines and instrumentations for this community

3. Identify R&D areas that will prepare the community to address grand challenge problems

# Non-Biological Membranes

Peter Pintauro ( Case Western) *Polymeric Membranes for Fuel Cells*

Jeff Brinker ( U of New Mexico and SNL)
*Self- Assembly of Biologically Inspired Complex Functional Materials*

Gerard C. L. Wong (U of Illinois UC) *Self-Assembled Complexes of Biopolymers and Charged Membranes*

Jin Wang (ANL) *Grazing-Angle X-ray Techniques for Studying Membrane and Ultrathin Films*

Miriam Rafailovich (SUNY Stony Brook) *Producing Low Density , Porous Polymer Films Using Supercritical Flu*

Michael Tsapatsis (U of Minnesota)*Molecular Sieve Membranes: Zeolite Films and Polymer Nanocomposites*

Giselle Sandi (ANL) *In Situ SAXS and GISAXS Studies of Polymeric Membranes for Energy Applications*

William J. Koros (Georgia Tech) *The Next Generation of Membrane Materials and Structures for Separation of Gas Mixtures with the Potential to Minimize Energy*

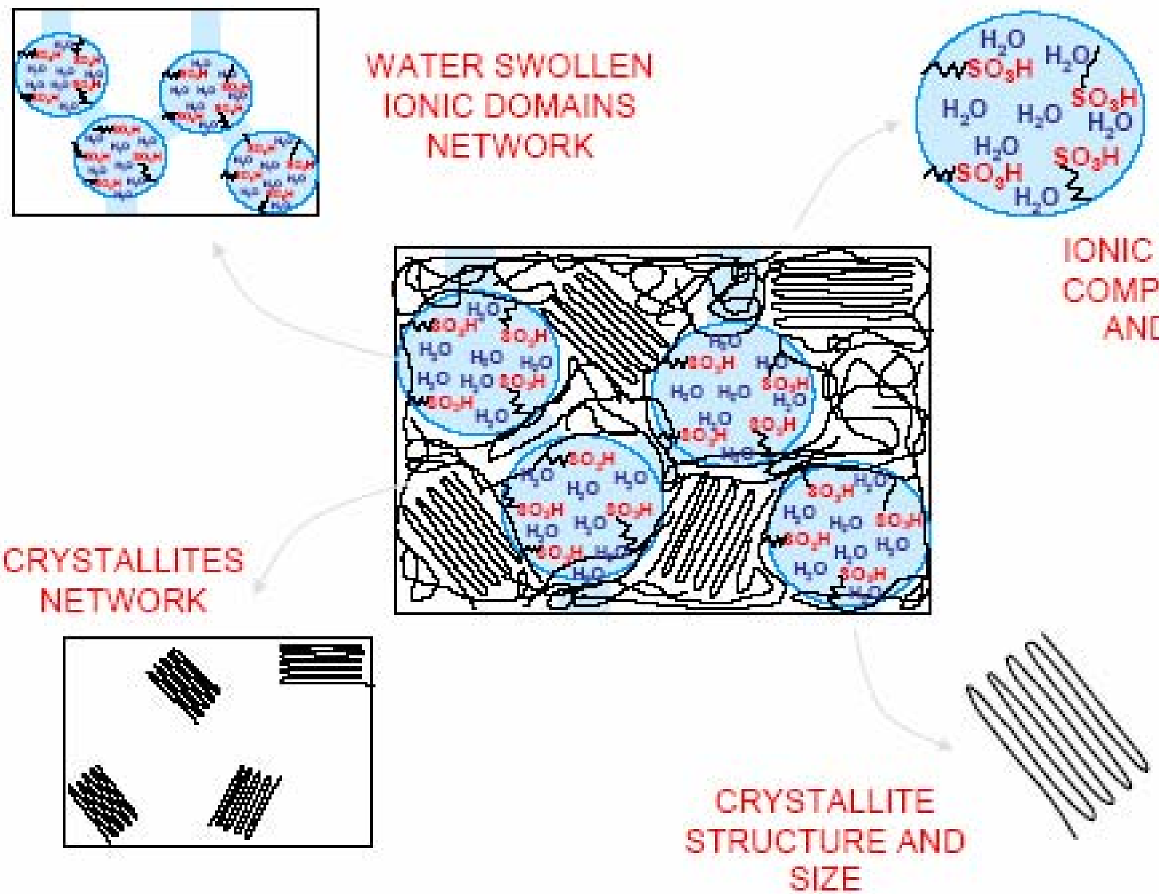
Larry Lurio (Northern Illinois U**)** *Use of X-ray Coherence to Study Dynamics in Thin Films, Layered Systems and Membranes*

# WORKSHOP ON MEMBRANE SCIENCE USING X-RAY TECHNIQUES

Polymeric Membranes for Fuel Cells:

Phase separated systems, buried interfaces
Peter Pintauro.

## Classical Copolymer Morphology (ionomeric domains in an inert semi-crystalline matrix) – Use X-Rays and/or Neutrons to Probe all Regimes

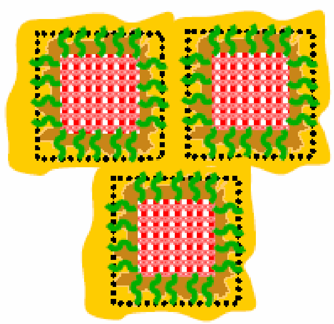



Microfocus and high energy needed to access buried interfaces.
Key advantage of APS. Need follow up workshops on buried interfaces.

The synthetic membrane community needs to better understand:
- ‘Buried interfaces’ between domains
- morphologies at multiple structural levels in complex structures
- the relationship of these factors to processing approaches

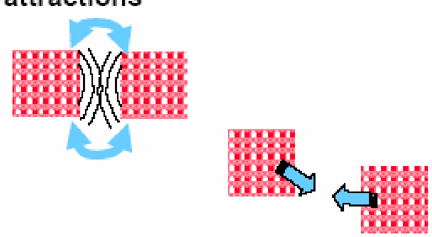
William J. Koros

## Key fundamental questions impact materials & spinning topics



- How are <u>sub</u>-nanometer level properties of flexible chain organic polymers affected near surfaces of rigid submicron solids?
  - transport properties in the *nanometer* domain near solid surfaces *versus* the bulk polymer “far” from the surface?
  - effective mechanical properties (modulus & transport properties) as “zones of influence of dispersed solids overlap in casting dopes & vitrified hybrid material ?

Van der Waals attractions

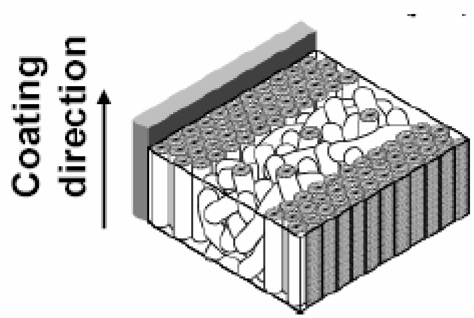


Collision-induced aggregations

- How do millisecond time-scale events during formation of asymmetric skinned structures affect properties noted above compared to the case of a “simple” solution cast dense film?

## Platforms to simultaneously characterize structure and function. Jeff Brinker.

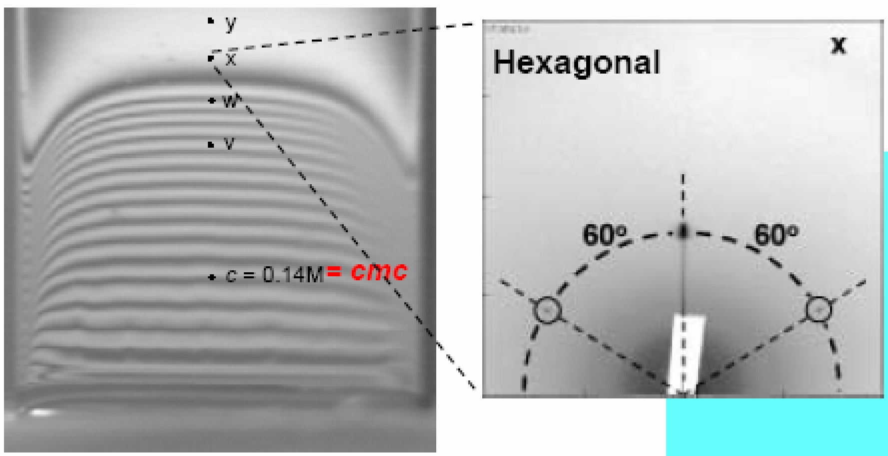
### *In situ* Characterization of Self-Assembly



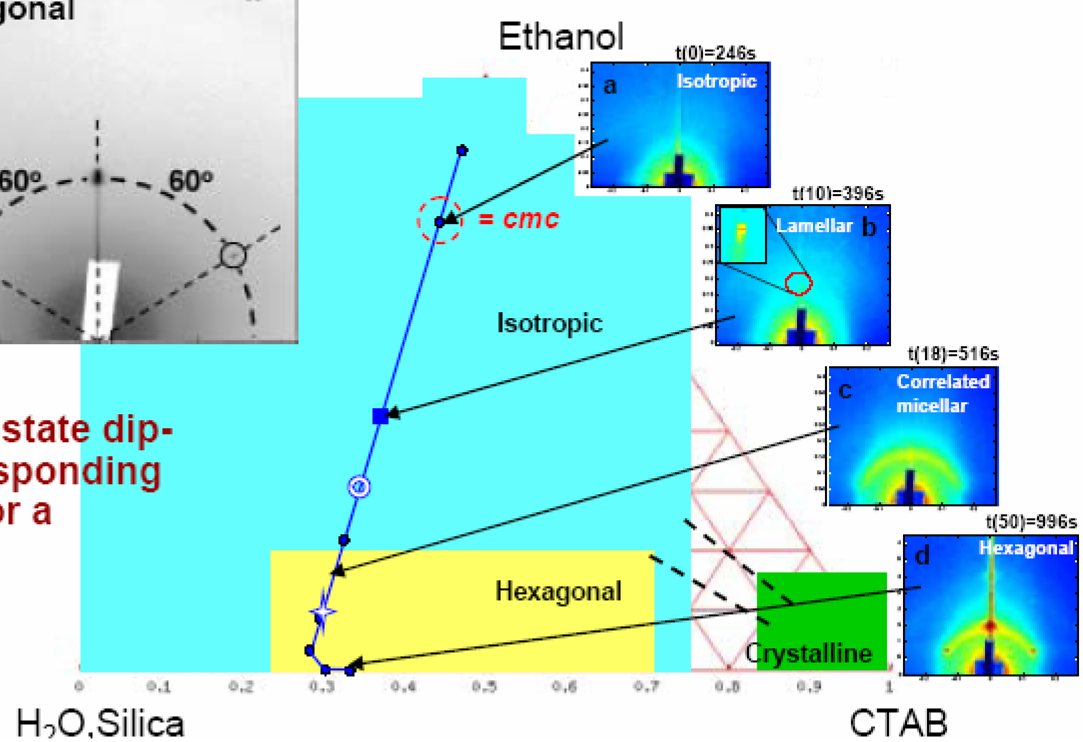


GISAXS patterns (a-d below) obtained using the liquid spectrometer at X22B, NSLS, Brookhaven National Laboratory (left) mapped onto the bulk CTAB/ethanol/water phase diagram

Doshi et al. *J.Phys. Chem. B*- *J.Am. Chem. Soc.* 2003

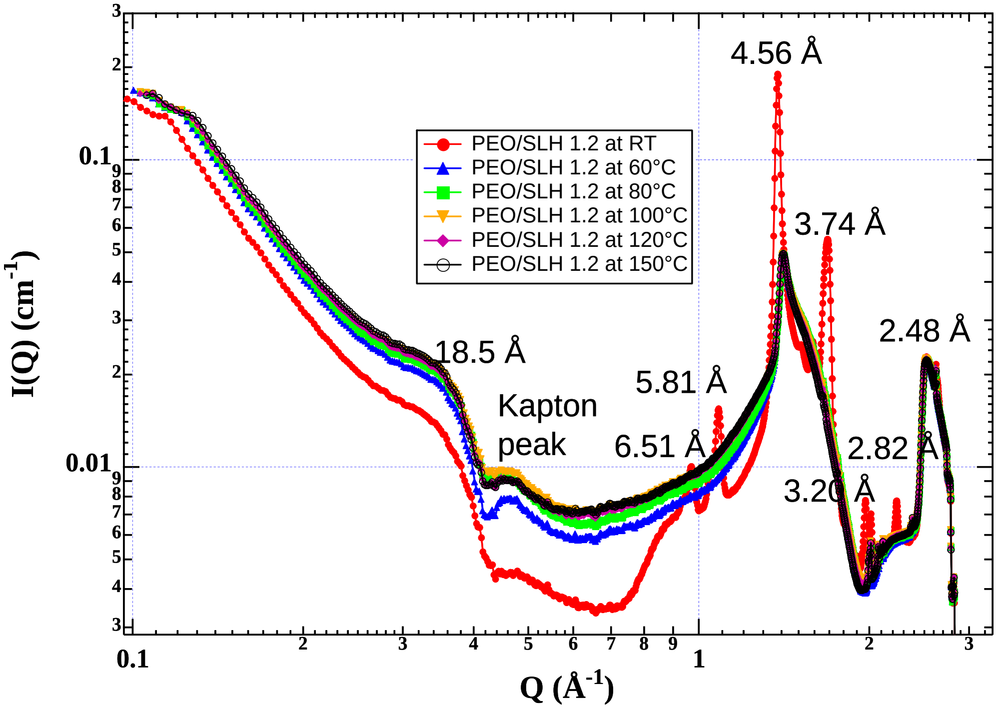



Optical interferograph of a steady state dip-coating profile (left) and the corresponding GISAXS pattern obtained *in-situ* for a hexagonal mesophase that forms via an interfacially mediated transformation of a correlated micellar intermediate (see schematic above)

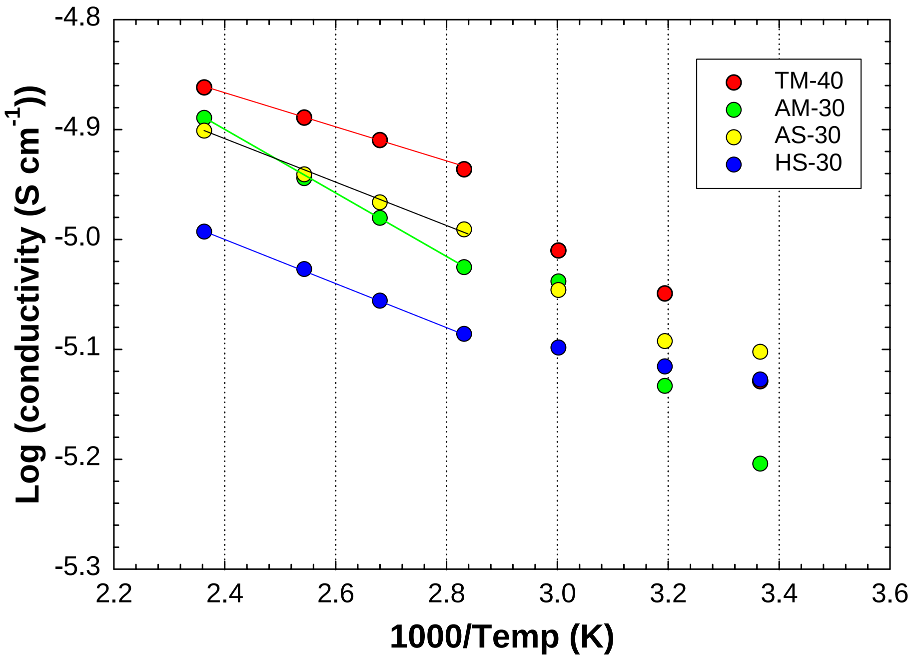


The polymer amorphous phase is responsible for the ion conduction in the polymer nanocomposite membranes. In situ SAXS is an excellent technique to follow these changes and correlate structure with performance. G. Sandí

***In Situ SAXS Data of a SLH:PEO 1.2:1 Ratio Film***




***Arrhenius Conductivity Plots Derived from Nanocomposite Films of PEO:Clay:1.2:1 Ratio***




Combine scattering, thermal treatment and conductivity measurements

## BioMembranes

Sol Gruner (Cornell University) –
*Some Unanswered Questions in Membrane Science*

John Nagle (Carnegie Mellon) –
*Diffuse X-Ray Scattering Provides More and Better Information about Membranes than Traditional Diffraction Methods*

Huey W. Huang (Rice University) –
*Biomembrane Problems Studied by X-ray and Neutron Diffraction*

Martin Caffrey (Ohio State University) –
*Membrane Structural Biology, Membrane Protein Structure*

Deborah Leckband (University of Illinois Urbana Champaign) –
*Molecular Design Rules for Biological Adhesion*

Mark Schlossman (University of Illinois Chicago) –
*New Methods to Study Biomolecules at Liquid Surfaces*

Lukas Tamm (University of Virginia) –
*Elastic Coupling of Membrane Protein Structure to Lipid Bilayer Forces*

Michael Kent (Sandia) –
*Protein Adsorption to Lipid Membranes through Metal-Ion Chelation Studied by X-ray and Neutron Reflectivity, and Grazing Incidence X-ray Diffraction*

Michael Kent (Sandia) –

*Protein Adsorption to Lipid Membranes through Metal-Ion Chelation Studied by X-ray and Neutron Reflectivity, and Grazing Incidence X-ray Diffraction*

KaYee Lee (University of Chicago) –

*Lipid Coralling and Poloxamer Squeeze-Out in Membranes*

Sue Pierce (NIH) –

*The Role of Membrane Microdomains in Immune Cell Signaling*

Adam Hammond (Cornell University) –

*Are You In or Out: Biological Rafts and Biophysical Phases*

Tobias Baumgart (Cornell University) –

*Coexisting Fluid Phases in Model Membranes and Biological Membranes*

Robert MacDonald (Northwestern University) –

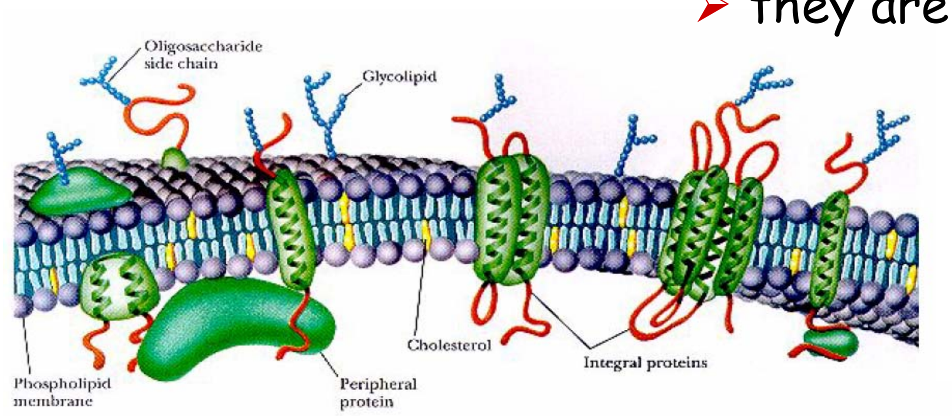
*X-ray diffraction in the Study of Cationic Phospholipid Derivatives: Lipoplexes, Lipid Mixtures and Bilayer Fusion*

Larry Scott (*Illinois Institute of Technology*, Chicago) –

*Lateral Organization in Lipid Bilayers: Atomistic and Coarse-Grained Simulations*

# Fundamental Questions in Biomembranes

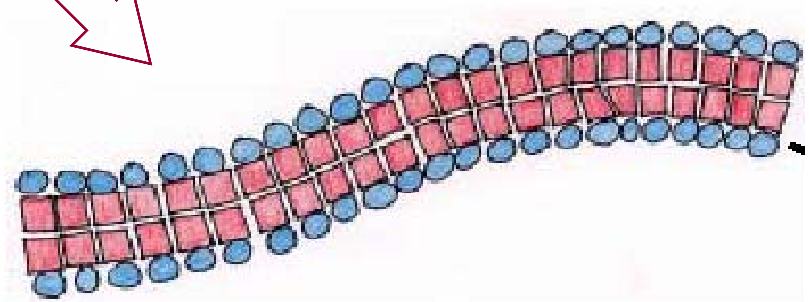
Cell Membranes
- compartmentalization of life
- they are complex, heterogeneous and dynamics




Garrett & Grisham

*"Natural biomembrane"*

*"Synthetic biomembrane"*



Many unanswered questions
- lack of suitable model systems
- requires multi-length scale characterization (need for many experiments & techniques
- significant opportunities for studying dynamics

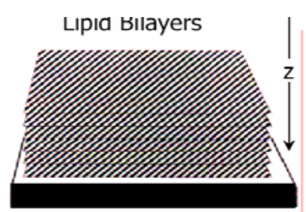
# Current Model Membranes

## I. Support lipid Monolayers

*LB – on trough or on a solid support*

- only a single leaflet of a membrane
- subphase or surface interactions?

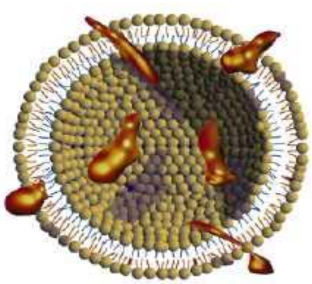
## II. Support lipid bilayers (Stacks)




*Dried onto a solid support and re-hydrated*

- truly hydrated?
- not a realistic model of a lipid bilayer
- interbilayer interactions?

## III. Multilamellar/ Unilamellar vesicles



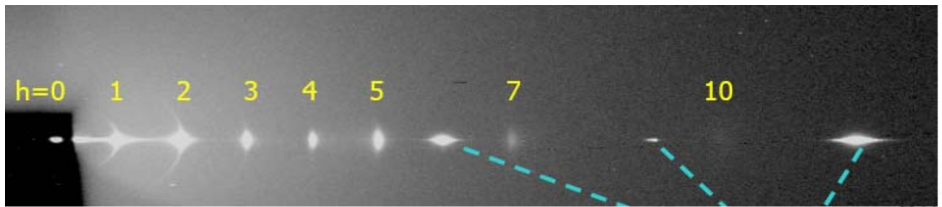
*Weak suspensions in solution*

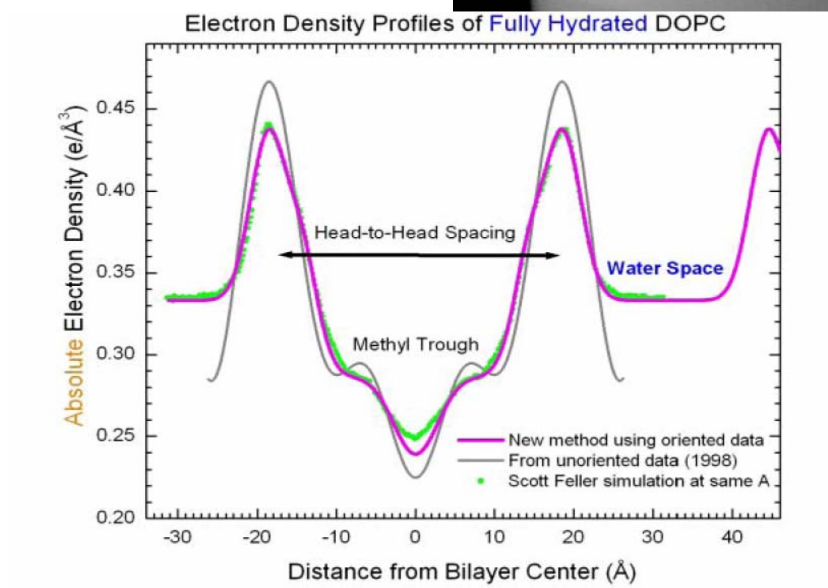
- polydispersity
- instability
- "powder" diffraction – loses information

**Challenge: Develop synthetic, model biomembranes that really mimic natural cell membranes**

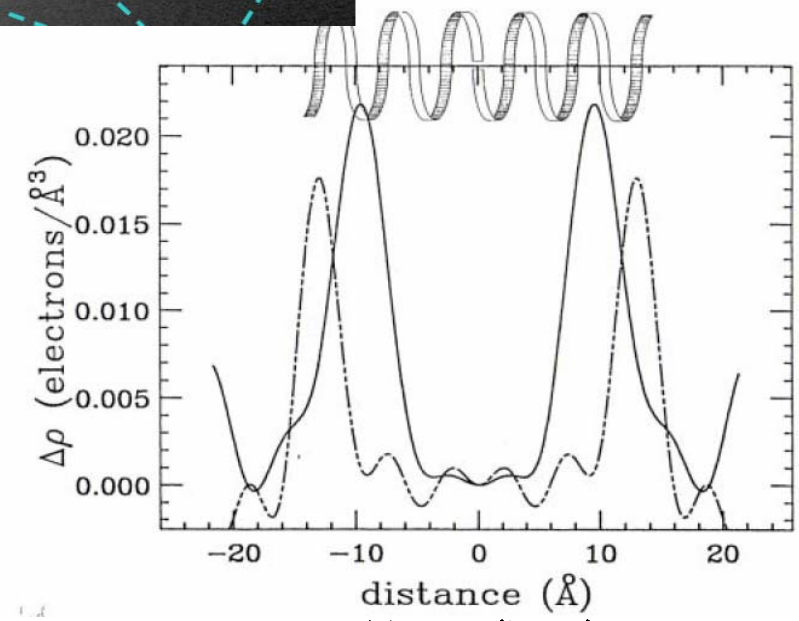
# Current Approaches to Membrane Structure

SAXD/ GISAXS – used to study the structure of supported lipid membranes
- static structures




Nagel (CMU)




Haung (Rice)

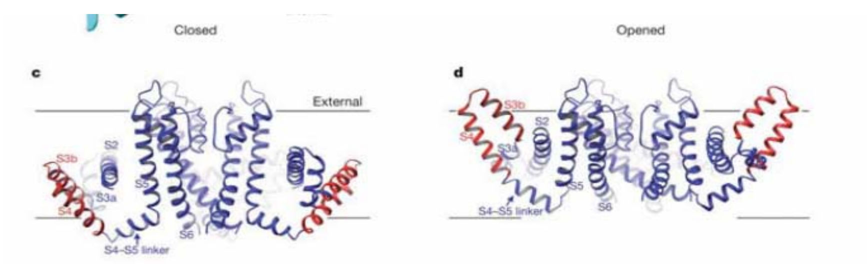
- o hydration layer
- o interbilayer interactons

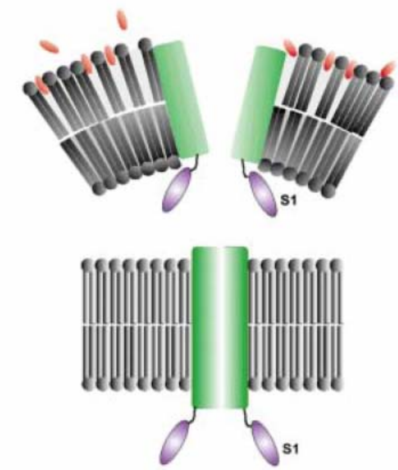
- o internal membrane ion-binding sites
- o guest intercalation (peptides)
- o transmembrane pores

# Membrane Protein – Lipid Interactions

Martin Caffery (Ohio) – rapid, high throughput single xtallography of membrane proteins
- NIH Grand Challenge-characterization of membrane proteins
- 27% of genome codes for integral membrane protein (~ 40,000)
- to data only the structure of 25 are known!

- ➢ What is the structure of integral membrane proteins in a biomembrane (vs crystals)?
- ➢ How does the membrane (micro)environment modulate protein structure/function?
- ➢ What is the structure and dynamics regulating membrane associated proteins (Cellular communication / signal transduction)?

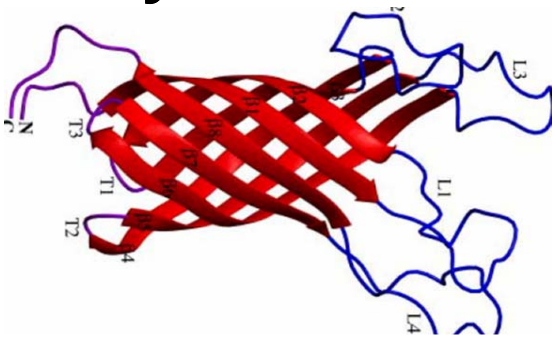


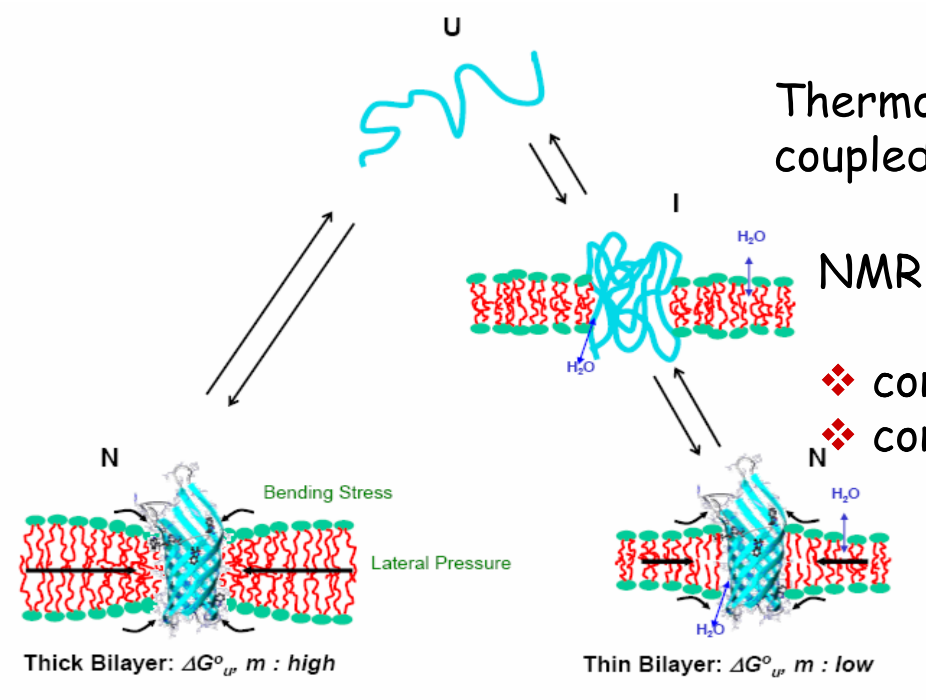

Mechano-activated channels

- ➢ Symmetric lipid bilayers don't occur in nature. How do we create a suitable model ?
- asymmetry in composition and electrostatics

# Dynamics of Integral Membrane Protein Insertion



OmpA
- structural protein connects outer membrane with periplasm
- ion-channel (anion selective)
- bacteriophase receptor
- mediates bacteria conjugation




Tamm (UVa)

Thermodynamics of folding/unfolding are elastically coupled to internal bilayer forces / pressure

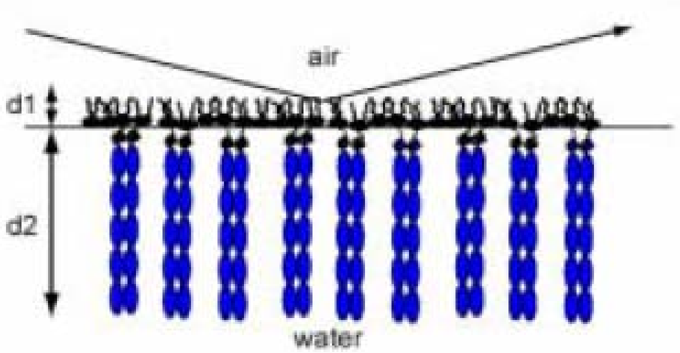
NMR Spectroscopy –both structure and dynamics

- conformational exchange μs – ms
- conformational domain dynamics ps- ns

**Challenge: X-ray techniques that allow for characterization of multi-time domain and length scale characterization**

# Membrane Structure - Function

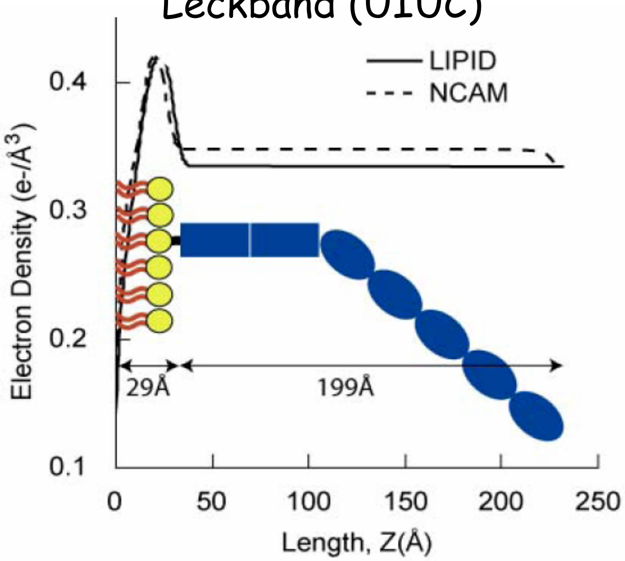
X-ray reflectivity and GIXD
– used to study the structure of on-trough lipid monolayers

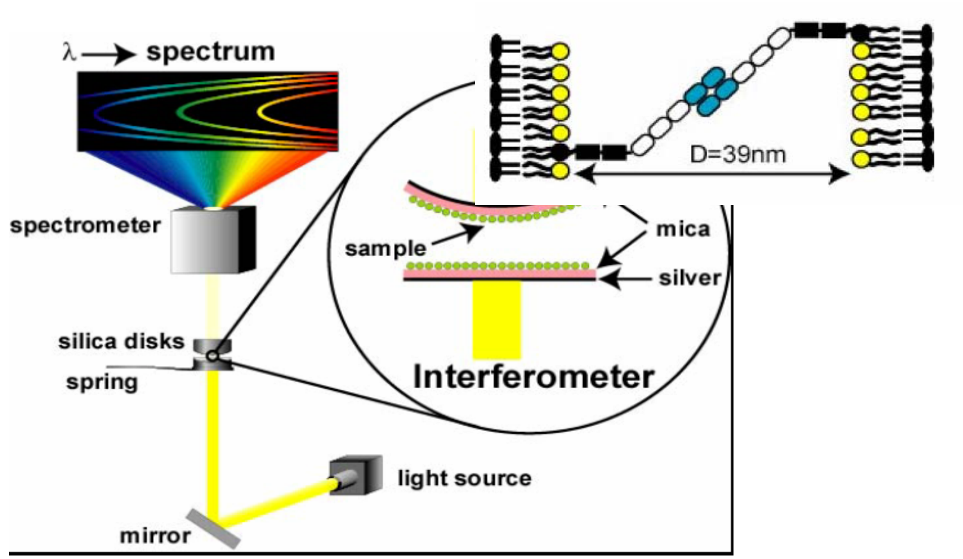



Structural studies of adhesion proteins

- Adhesion complexes regulates intracellular space
- important for signaling / cellular communication

Leckband (UIUC)

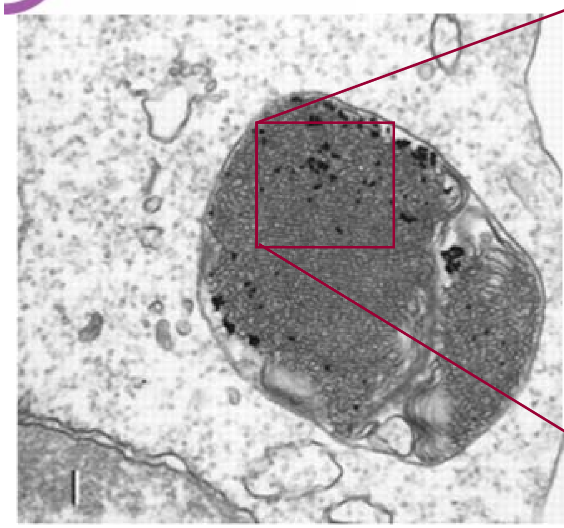


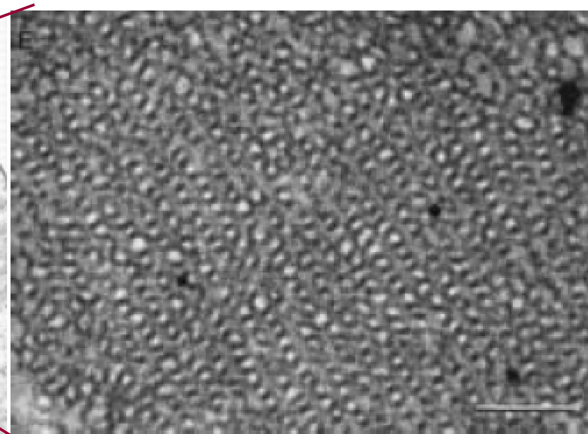




SFA – used to determine energetics of binding

**Challenge: In-situ, combined experiments**

# Polymorphism of lipid phases





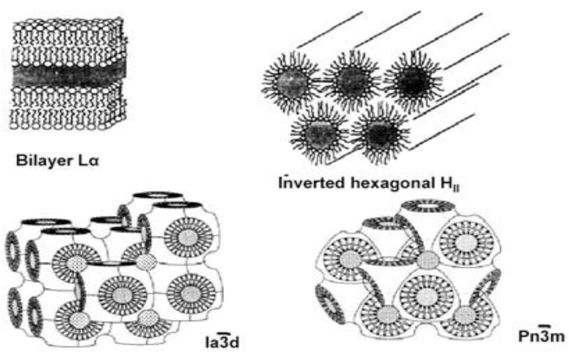
MacDonald (Northwestern)

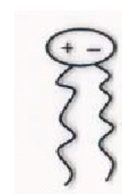
Does nature use non-planar (bilayer) membranes?

Why?

*Bilayer cubic arrays have been observed in lipoplexes internalized in cells*

- A broader perspective comes from the study of "complex fluids"
- Self-assembly of amphiphiles in water (surfactants, polymers, etc.)

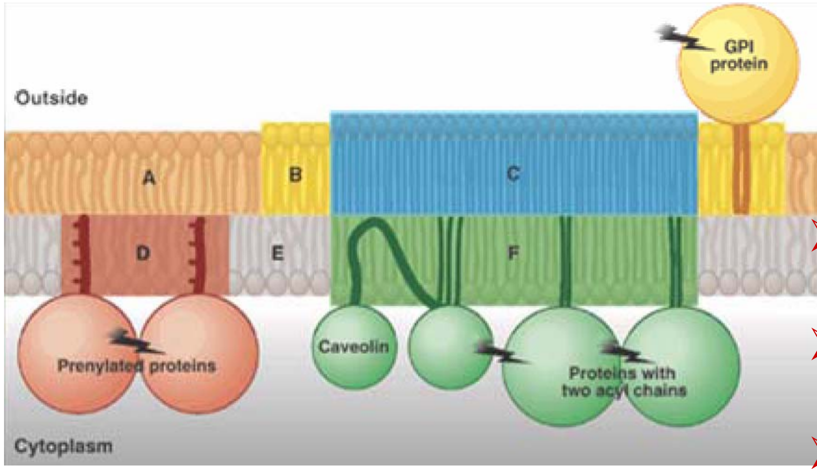


- Given a chemical structure of an amphiphile can we predict the phase diagram?
- Does nature use lipid composition to regulate local structure/curvature ?

**Challenge: Unlock the fundamental rules of amphiphile self-assembly**
**Structure = Function**

# In-Plane Structure – Lipid Rafts

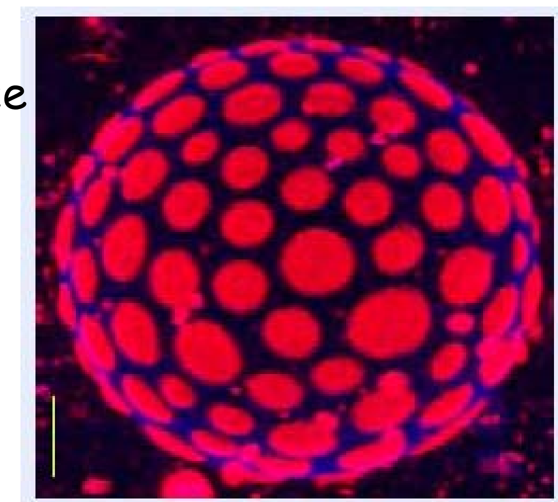


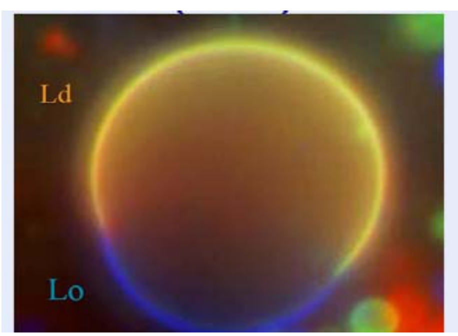

**Recent recognition that the lateral organization of lipids many NOT be isotropic**

- in-plane co-existence of lipid patches of different compositions.
- biochemically distinct region within a continuous lipid bilayers.
- why is this biologically important? Signaling/ trafficking

2-photon confocal fluorescence microscopy technique of choice

- non-destructive
- optical sectioning
- domain shape and line tension







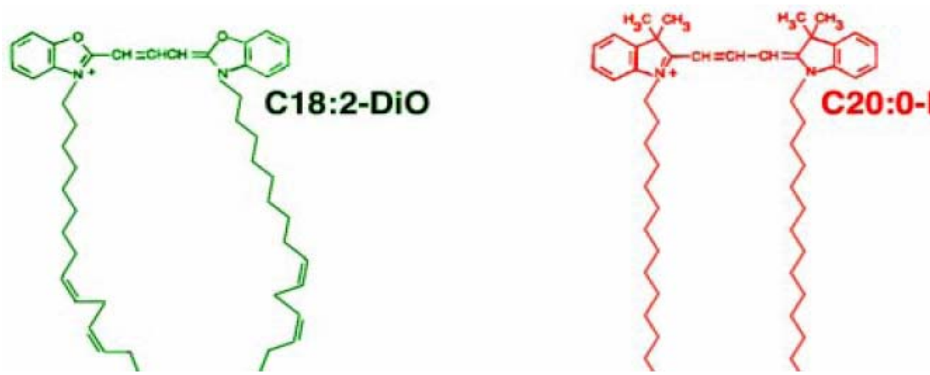
*Tobias Baumgart/ W. Webb (Cornell)*

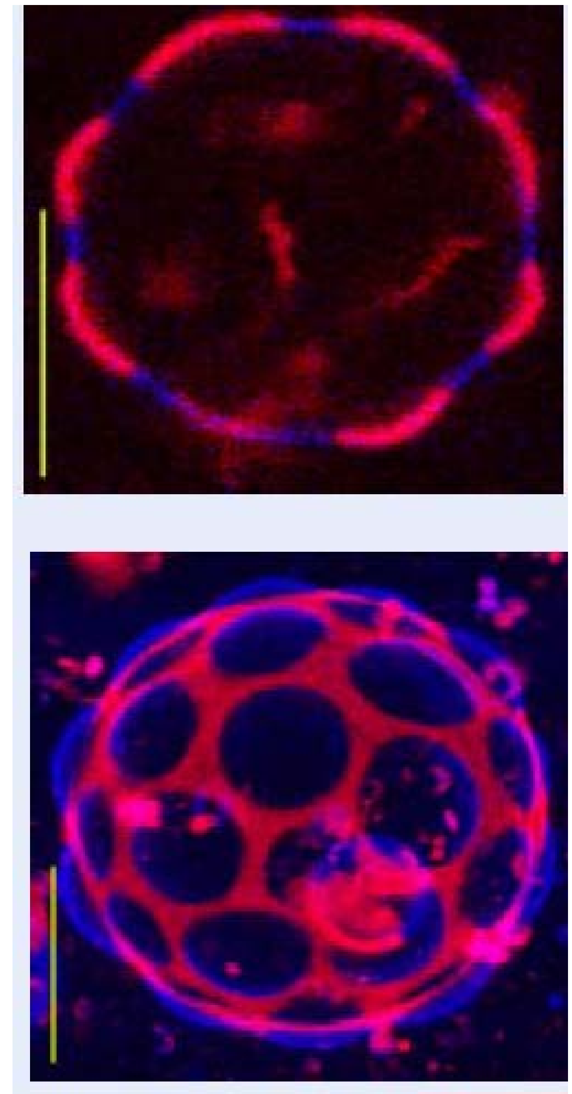
Challenge: X-ray techniques that can probe in-plane structure (imaging, focused beams)

# Opportunities for the Application of X-rays to the Study of Lipid Rafts

Confocal microscopy requires use of dye-labeled lipids

- how do these bulky dye moeities modify phase behavior
- do they artificially promote segregation?



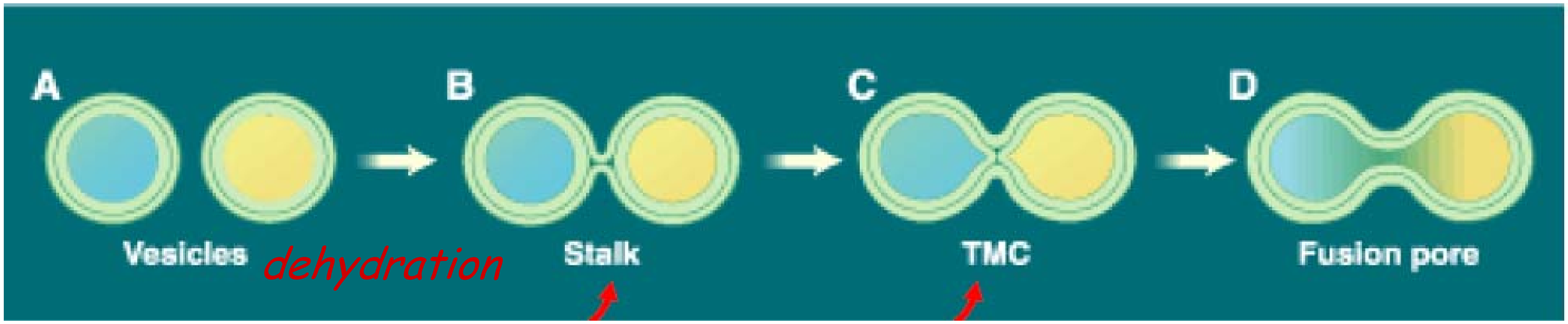



No reports of the use of X-rays to probe lipid rafts

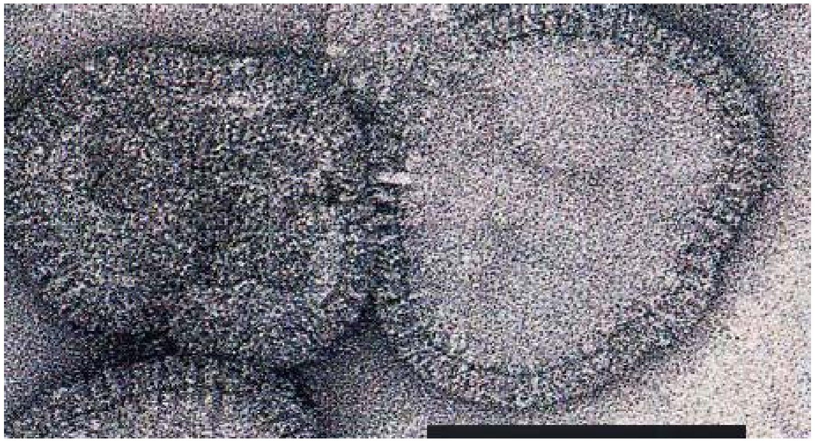
Grand challenge – focused beams
- new approaches

**Is it real or an artifact?**

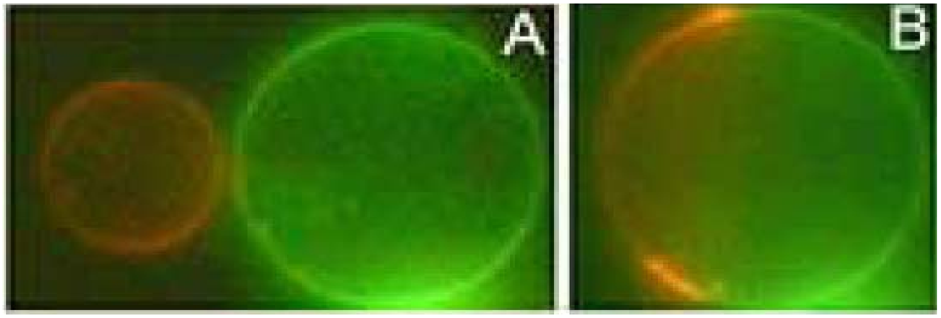
# Membrane Fusion




Fusion = the greater tendency of lipid membranes to generate high curvature phases
Important for delivery of drugs, viral agents, DNA (gene therapy) and understanding endocytosis



Cryo-TEM




Confocal Fluoresence Microscopy

*MacDonald (Northwestern)*
*Haung (Rice)*

**Challenge: X-ray techniques that in-situ characterize curvature and fusion events**

Challenges

# Grand Challenges In Membrane Science and Opportunities for X-ray Techniques to Address them

# Scientific Challenges

**Bio-membranes: Self-organized lipids and proteins**
Interactions to function, to self-assemble, to transform, to react
Thermodynamics of the assembly
Controlled release and drug delivery
?????

**Organic-membranes: Polymeric matrix and composites**
Nature of Hydrophilic/hydrophobic interaction for fouling
Specific and nonspecific adsorption and absorption
Porosity control
?????

**Inorganic-membranes: Inorganic metal or ceramic alloys and nanocomposits**
Materials synthesis and characterization
Membranes for hydrogen production and separation
Catalytic activities
?????

**Challenges: Effective Methods of Characterizing Membranes**

# Technical requirements

Fundamental and understanding is necessary to control the formation of membranes on all length scales and time scales

**X-rays offer nondestructive and high-resolution and *in situ* characterization**

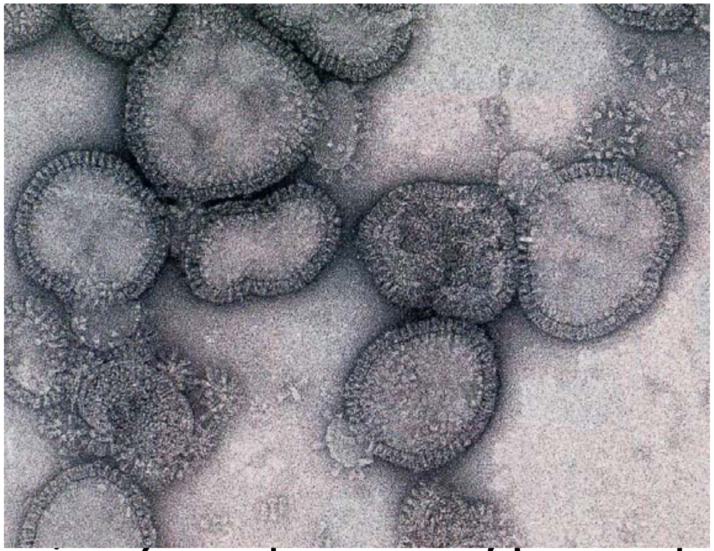
1. General techniques and instrumentations:
   small-angle x-ray scattering (SAX), powder diffraction, EXAFS
2. Surface sensitive techniques:
   x-ray reflectivity (XRR), grazing incidence small-angle scattering (GISAXS), x-ray standing wave (XSW), GI diffraction (GID)

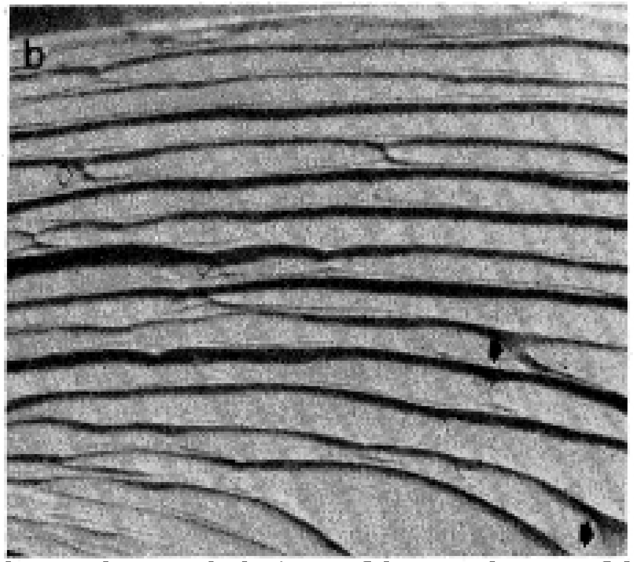
These are currently available at the APS at various beamlines.
They have to be tailored to meet the need of the membrane community.
(see below)

# Non-Conventional techniques

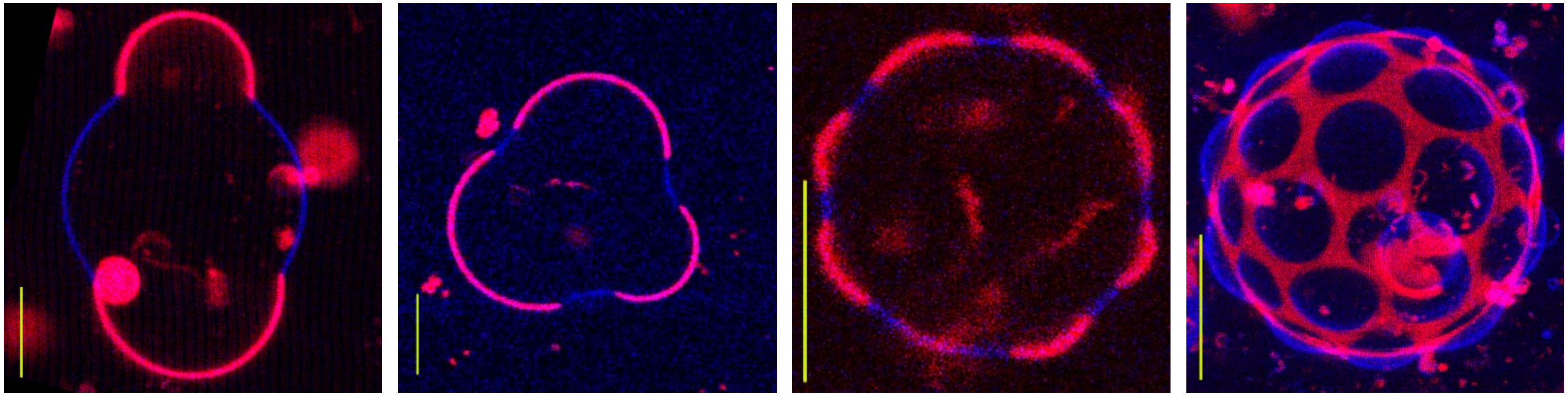
## Microfocused beams

Imaging (membrane fusion, inorganic membrane textures)





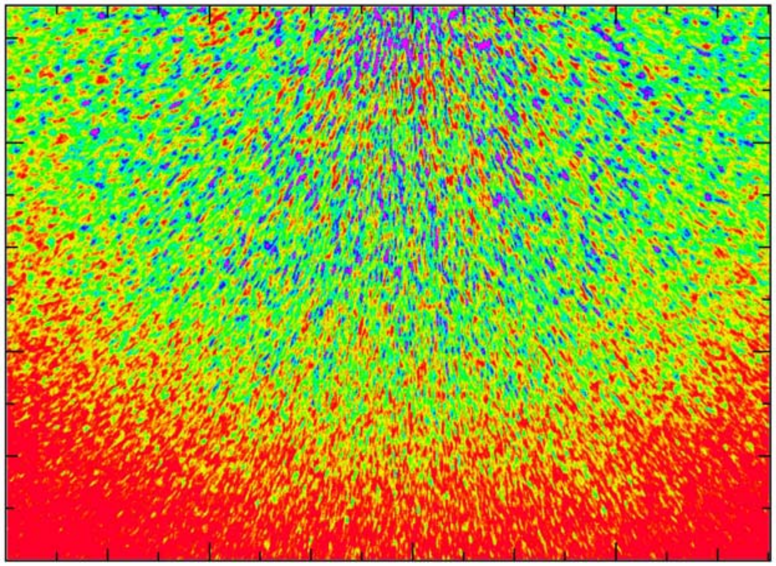

Scattering (membrane raft, membrane localized fine structures)

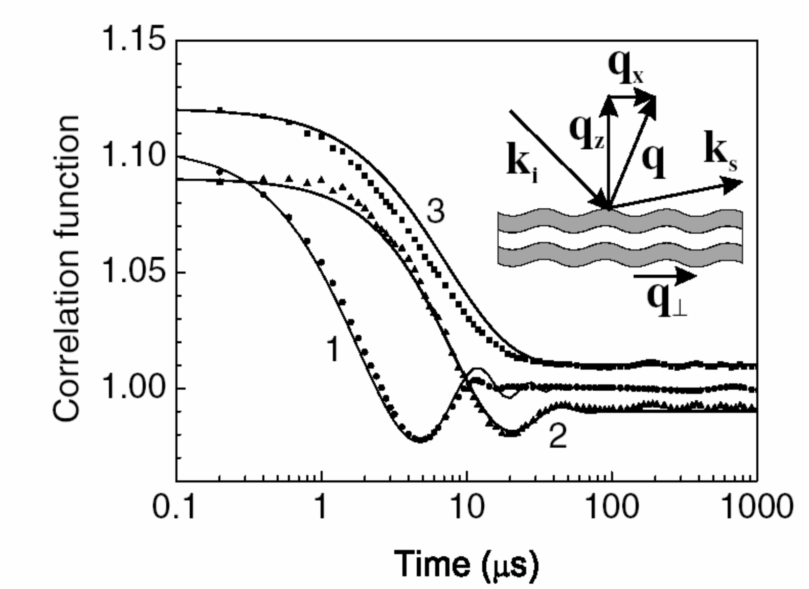


# Non-Conventional techniques

## Dynamics of Membranes

- ✓ Dynamics of membranes in equilibrium have never been probed by any techniques.
- ✓ X-ray photon correlation spectroscopy using coherent x-rays should shed light to the dynamics



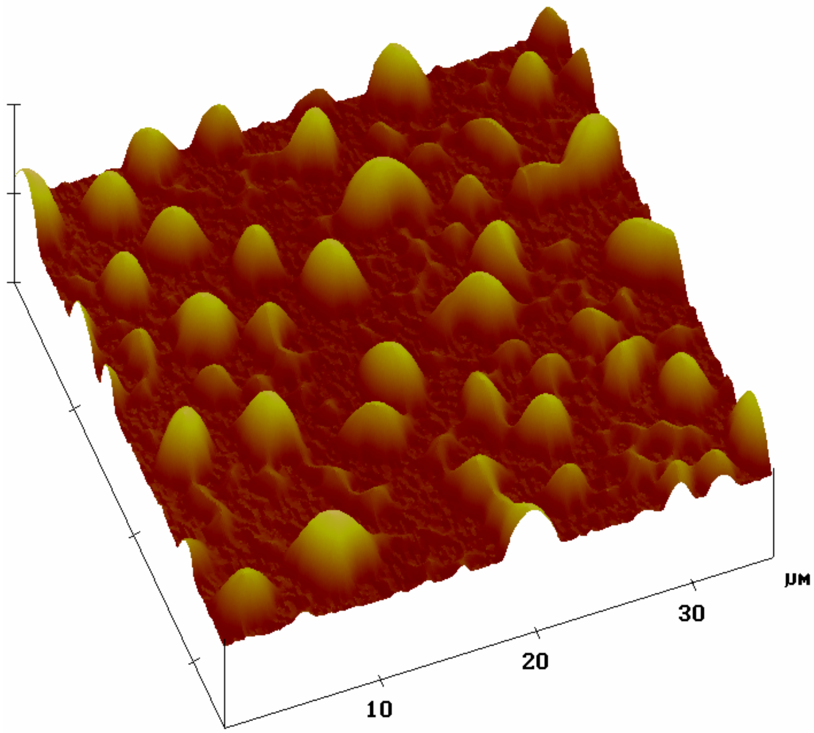


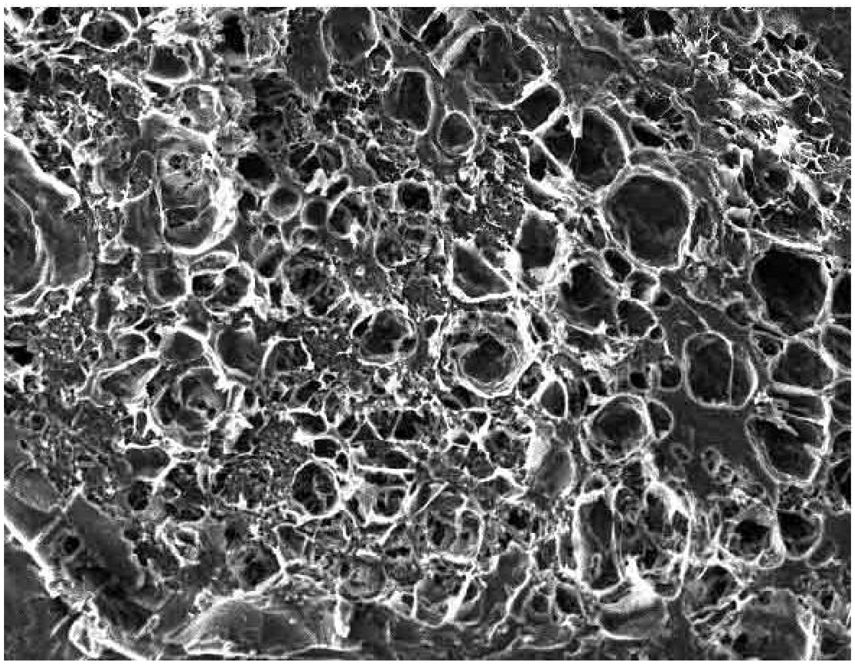

- Biomembranes dynamics: lots of challenges: fast dynamics, damage, but possible in next 5 years.
- Extend measurements to larger in-plane wavevectors at faster times.
- Fast, efficient detectors

# Dedicated Beamlines and instruments

**Co-location of conventional instrument at the beamlines**

- Optical microscopes (fluorescence, etc)
- Various spectrometers
- NMR
- Atomic force apparatus
- SPM



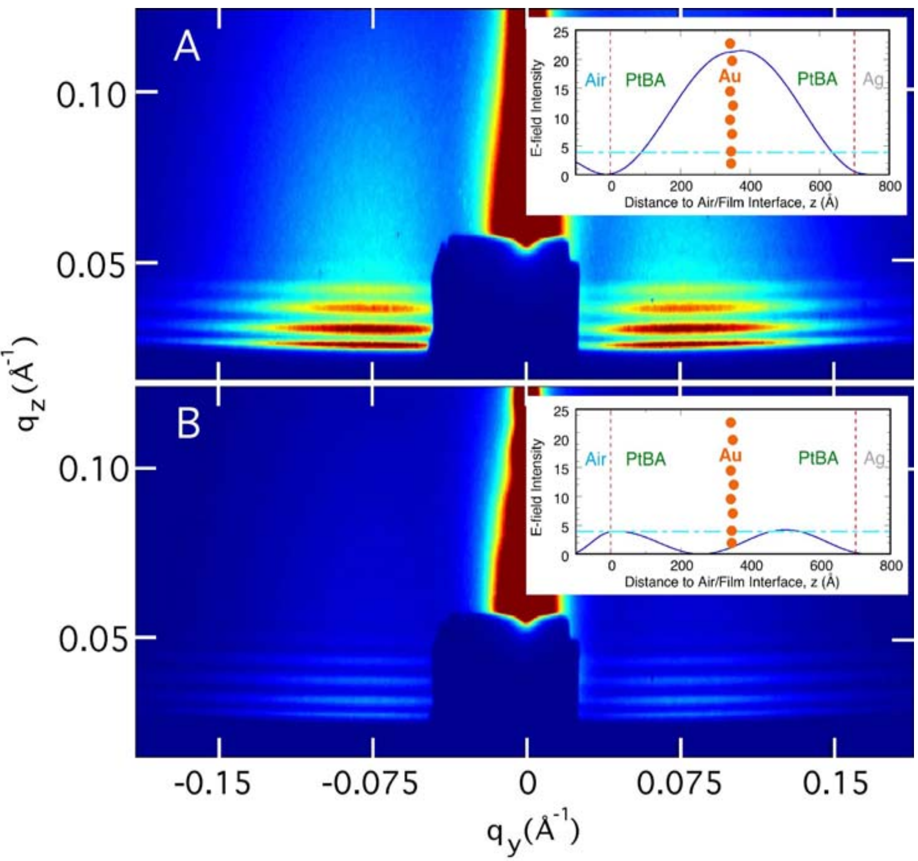



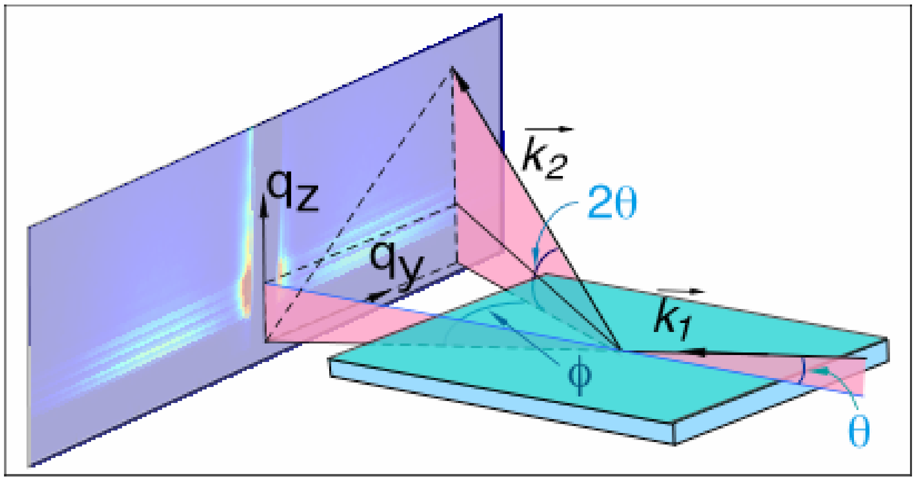
Off-line instrument but readily accessible, TEM, SEM etc.

# Dedicated Beamlines and instruments

## GISAXS Beamlines

- A guided formation of membrane requires a thorough understanding of kinetics of self-assembly




- **Both bio- and nonbio-membrane communities expressed STRONG interest in the in situ, real-time and nonintrusive probe!**
- **Dedicated GISAXS beamlines are needed!**

# R&D to Address the Challenging Problems

## General R&D

**Maintain and cultivate "in-house" expertise in areas of interest**

- Difficult but essential to attract and support users
- Non-biomembrane community by large has not been aware of APS

**Need significant improvements in software for data analysis**

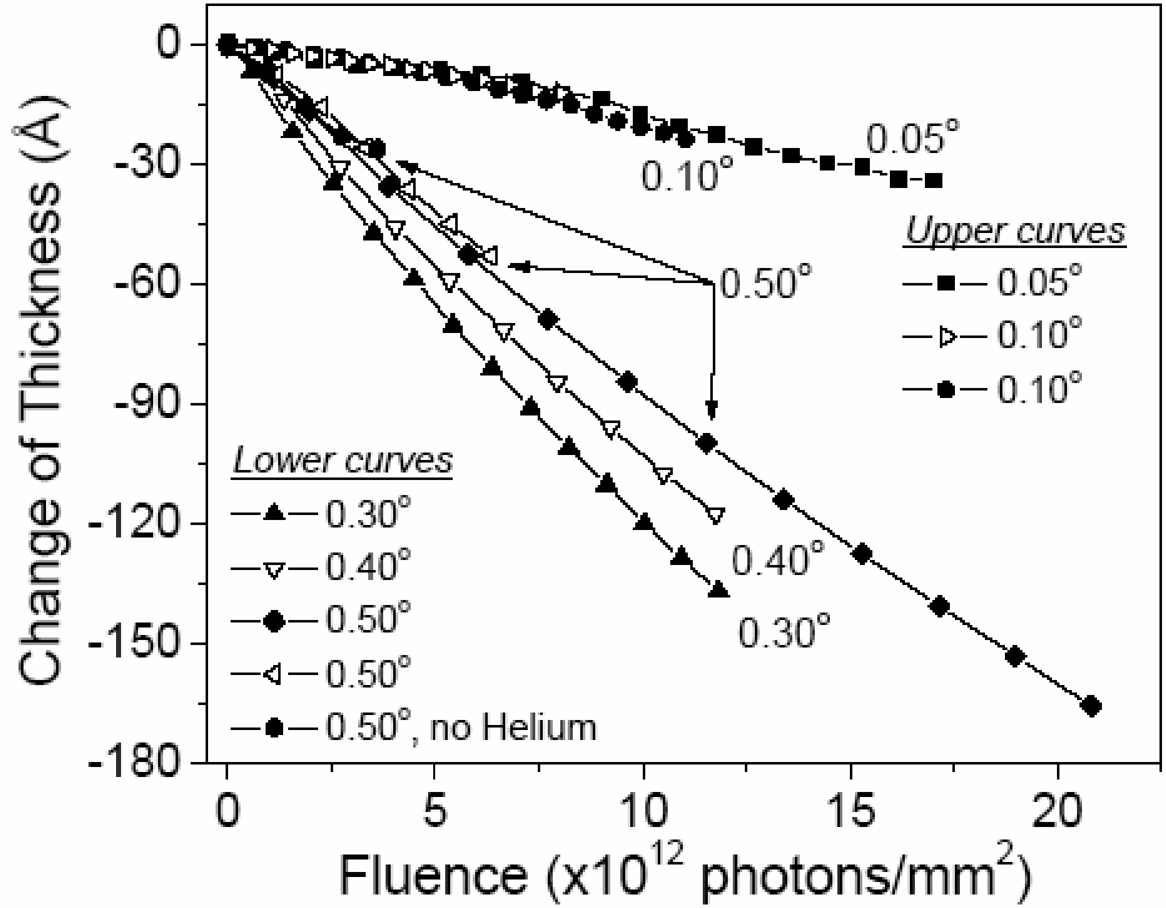
- Critical for user not coming from physics community
- User friendly
- Reflectivity, small-angle scattering, GISAXS…

## Detectors!

Highly sensitive and efficient - samples are to precious to waste
Time resolved for kinetics studies
Ultra low noise for weak scattering from soft matters
How about a digital pixel array detector? (1MHz, no noise, fast framing)

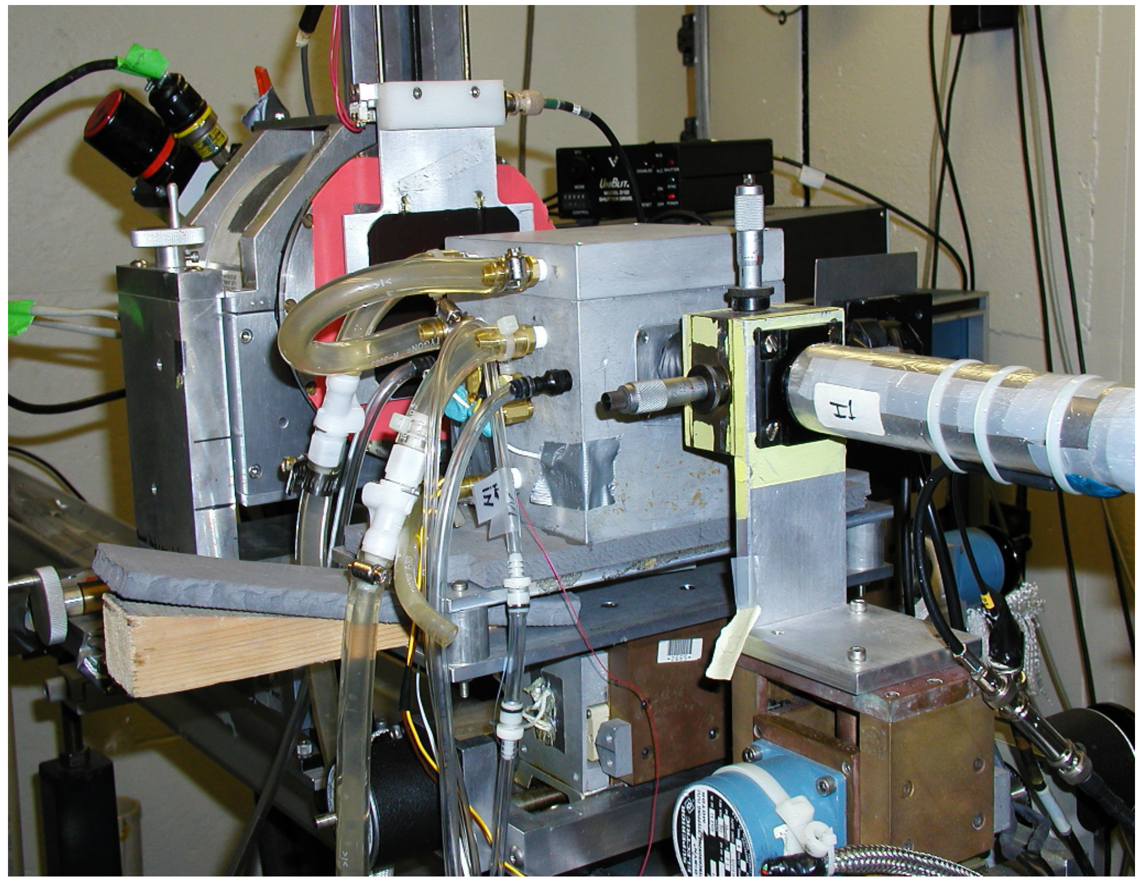
# R&D on Radiation Damage issues

- These are the experiments no one wants to do
- Need for a systematic study
- How do we solve the problem – go beyond cooling the sample – improved detectors or optics?
- A low background beamline



# R&D on Sample Environment Issues

- Aqueous environment
- Controlled atmosphere (humidity, gas, etc.)
- In situ and simultaneous thickness measurement
- External fields (electric, magnetic)
- Controlled temperature
- Pressure
- Balance



# Cultural Change

## From a "physics"–style experiment to a "biochemistry" experiment

- Need lots of data (controls, parameters): can't go after a PRL with one experiment.
- sufficient / or appropriate allocation of beamtime to carry out systematic studies
- Sufficient support at beamlines (staff, technique, instrumentation)

**X-rays alone won’t provide the answers to these (membrane) questions – the best information comes from combining different experimental approaches.**

**But it is clear that many x-ray techniques, in the future as in the past, will be at the forefront in investigating these (membrane) questions.**

- Sol Gruner (Cornell University)